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Nothing to declare

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- **Activation of the inhibitory receptor LIMR3 on MC reduces colitis**
- **The MC proteases MCP-6 and Prss31are involved in DSS and TNBS colitis**
- **Protease inhibitors or LIMR3 agonists could be interesting compounds to treat IBD**

Mast cells and inflammatory bowel disease

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Abstract (100-120 words)

Inflammatory bowel diseases (IBD), consisting of ulcerative colitis (UC) and Crohn's disease (CD), are chronic immune-mediated diseases of the gut. Here, the potential role of mast cells (MC) is discussed, mainly focusing on preclinical studies. MC can be activated by antigen-mediated crosslinking of immunoglobulin receptors, by free light chains of immunoglobulins, stress and ATP. Upon activation, MC release bioactive mediators, of which the serine proteases mMCP-6 and Prss31 were shown to be involved in the development of acute colitis. Inhibition of MCs by activation of the inhibitory receptor LIMR3 or inhibitors of proteases may therefore represent new therapeutic targets to treat IBD. Human data are however lacking.

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Introduction

Crohn's disease (CD) [1] and ulcerative colitis (UC) [2] are the two main types of idiopathic chronic inflammatory disorders (IBD) of the gastrointestinal tract, both associated with a significant impact on the quality of life of the affected individuals. While CD can affect any part of the gut, albeit with a predilection for the terminal ileum, UC rather typically starts in the rectum and may extend all the way up to the caecal area. Despite extensive scientific effort, the exact underlying cause of IBD remains unclear. It is however becoming increasingly clear that a dysregulated mucosal response to the commensal gut flora is involved, in particular in genetically susceptible individuals. In UC, this immune response involves an atypical Th2 response mediated by non-classic natural killer T cells. In contrast, in Crohn's disease, imbalance between predominantly Th1 or Th17 cells and naturally regulatory T cells is proposed to underlie the mucosal inflammation and associated damage. In the present review, the evidence, albeit scarce, supporting a potential contribution of mast cells will be summarized. Mast cells are particularly interesting as they are not only involved in the immediate and delayed defense against foreign antigens [3], but also release a mixture of mediators known to affect mucosal barrier function[4-6], thereby increasing the access of intraluminal antigens to the mucosal immune system.

Mast cells: general aspects

Mast cells arise from hematopoietic progenitors in the bone marrow that in contrast to most other immune cells only mature when they arrived at their final destination, i.e. all vascularized tissues but especially the skin and mucosal tissues. Typically, mast cells reside in the mucosa at anatomical sites directly exposed to the environment, such as the skin, the airways and the gastrointestinal tract, where they are ideally positioned to respond to foreign antigens that have crossed the epithelial barrier[6]. Their number can vary significantly depending on the tissue studied but also during infections or

inflammation. Both in man and mice, two main types of mast cells can be identified, largely based on location and protease content. In mice, connective tissue mast cells and mucosal mast cells are distinguished. Connective tissue mast cells reside mainly around venules[7] and nerve fibers and in serosal cavities. Mucosal mast cells on the other hand are present in the mucosa of the gut and lung, but in low numbers[8]. In humans, mast cells are characterized by the proteases they contain: the MC_T–type mast cells containing tryptase but little or no chymase, and the MC_{TC}-type mast cells containing tryptase and chymase[9]. Almost all human mucosal mast cells are MC_T while MC_{TC} are typically located in the lamina propria and submucosa.

Mast cells can be activated by a wide variety of stimuli. The most well known mechanism of activation is cross-linking of the high affinity IgE receptor FcεRI. This receptor binds antigen-specific IgE antibodies captured from the circulation by mast cell processes extended across the vessel wall [7]. Binding of these antibodies with their respective antigen leads to crosslinking and subsequently aggregation of FcεRIs thereby activating downstream signaling pathways and triggering the release of biologically active mediators [10]. In addition, mast cells can be activated via toll like receptors, G-protein coupled receptors, various complement peptides and platelet activating factor, illustrating its crucial role in immune defense [3]. Of note, mast cells are armed with granules containing preformed and stored mediators, such as vasoactive amines like histamine, and proteases such as tryptases and chymases, enabling an immediate response. In addition, mast cells can synthesize and release lipid mediators such as prostaglandins and leukotriens within 1-2 hours after activation. Mast cells also release cytokines and chemokines, a process that typically occurs later and is associated with more chronic responses. The wide range of mediators released undoubtedly underlie the many different functions of mast cells and their involvement in a diversity of mechanisms, including secretion and epithelial permeability, immune cell recruitment and activation, blood flow, coagulation and vascular permeability, wound healing and

fibrosis and neuro-immune interaction altering peristalsis, bronchoconstriction and pain [11].

Evidence in humans supporting a role for mast cells in IBD

To date, only few studies have evaluated the potential involvement of mast cells in IBD, and most of them date back many years ago and are rather descriptive reporting on the number of mast cells. Gelbmann et al. [12] for example describe accumulation of mast cells particularly in hypertrophied and fibrotic muscularis propria in strictures of CD patients, suggesting an important role in the process of fibrosis. Others report on increased mast cells in the mucosa, submucosa, muscularis propria and surrounding fat in active CD [13]. More recently, increased mast cell numbers were counted in the colon of both CD and UC patients compared to controls [14] [15]. Activation of mast cells on the other hand is suggested by increased expression or release of mast cell mediators in the mucosa of IBD patients [16] [17] or by demonstration of increased levels of the N-methylhistamine, one of the metabolites of histamine, in the urine of IBD patients compared to patients in remission and controls [18]. Although the above-mentioned observations may suggest a role for mast cells in IBD, this evidence is rather indirect and could also be secondary to the inflammatory response in the mucosa. The ultimate proof, i.e. clinical improvement by treatment with mast cell stabilizers or antagonist blocking the biological effect of mast cell mediators, is however currently lacking.

Mast cells and colitis: preclinical data

Several models of colitis are routinely used that can largely be divided in models of acute colitis[19], mostly due to exposure of the intestine to compounds damaging the intestinal barrier (dextran sulphate sodium (DSS)) in drinking water, enema of trinitrobenzene dissolved in alcohol) or more chronic models such as T cell transfer colitis or delayed-type hypersensitivity colitis (TNBS, dinitrofluoro-benzene). Clearly,

the role of mast cells in colitis will largely depend on the type of model studied. Below, recent new data supporting a role for mast cells in colitis will be briefly summarized according to the type of colitis.

Acute colitis

Previous studies have yielded conflicting data regarding the role of mast cells in acute colitis [20]. Kurashima et al. however nicely demonstrated a pivotal role for ATP-mediated activation of mast cells in DSS and TNBS colitis [15]. These authors demonstrated that treatment of mice with an antibody against the ATP receptor P2X7 inhibits mast cell activation and subsequent intestinal inflammation. Moreover, mast cells expressing P2X7 purinoceptors in colons of mice with colitis and of patients with CD are increased in number. The most compelling evidence however is revealed by reconstitution experiments in mast cell deficient $Kit^{W\text{-}sh/W\text{-}sh}$ mice. Intestinal inflammation triggered by DSS or TNBS in these mutant mice is ameliorated while reconstitution with wild type but not P2X7-/- mast cells results in susceptibility to develop colitis in response to DSS or TNBS [15]. Moreover, the authors showed increased ATP production in intestinal inflammation and demonstrated that ATP triggers MC activation through P2X7 purinoreceptors. Taken together, these data indicate that tissue damage induced by DSS or TNBS leads to ATP release subsequently activating MC by interacting with P2X7 receptors. These data reveal a role for MC in both the initiation and exacerbation of intestinal inflammation [15]. Of interest, the same group very recently showed that this pathway can be blocked by activation of the inhibitory receptor CD300f (also called leucocyte mono-immunoglobulin-like receptor 3 or LMIR3) on mast cells by its ligand ceramide [21]. Mice deficient for LMIR3 developed more severe DSS colitis. Moreover, reconstitution of mast cell deficient mice with LMIR3-/- MC exhibited more severe colitis than mice that received wild type MC. Previously, the authors have shown that ceramide is a ligand of LIMR3 leading to

inhibition of IgE-dependent MC activation [22]. In their recent paper, they take this finding further and show that ceramide-LIMR3 interaction not only inhibits ATP-induced MC activation but also suppresses DSS colitis. These data are of great interest indicating LIMR3-targeted compounds such as ceramide as a novel therapeutic strategy for IBD [21].

In mice, mast cells store a variety of proteases on their secretory granules. These enzymes are of particular interest as they are involved in proteolytic processing of other molecules but can also act as signaling molecules by cleavage and activation of Protease-activated receptors (PARs) [23]. The latter are abundantly expressed and trigger a wide array of pro-inflammatory processes upon activation, including colitis[24]. Two recent papers have studied the role of mast cell serine proteases in DSS colitis, in particular mouse MC protease-6 (mMCP-6), mMCP-7 and protease serine member S31 (Prss31) [25,26]. Hamilton et al. [25] elegantly showed that mMCP-6 null mice exposed to DSS had significantly less weight loss as well as lower pathological and endoscopical disease scores compared to mMCP-6 expressing mice. In line, the levels of the pro-inflammatory cytokines IL-6 and Il-1b and the chemokines CXCL1 and CXCL2, known to attract neutrophils, were significantly reduced in the mMCP-6 null mice. Of interest, similar findings were obtained in the TNBS colitis model. In a subsequent study, the same group showed that also Prss31 null mice lost less weight, had lower histopathological scores and contained less CXCL2 and IL-6 mRNA in their colons compared to WT mice following treatment with DSS. Taken together, these data provide strong evidence that both mMCP-6 and Prss31 contribute to the development of colitis and thus may represent interesting new targets for treatment of IBD [26].

Delayed-type hypersensitivity colitis

After sensitization, re-exposure to oxazolone, TNBS or dinitrofluorobenzene leads to a delayed-type of colitis. Classically, MC are activated by antigen mediated crosslinking of

membrane bound IgE antibodies. Recently, Hoving et al. [27] provided evidence for IgE-mediated activation of MC in oxazolone colitis. In vivo neutralization of IgE protected mice from oxazolone-induced colitis, a finding associated with a reduction in mast cell numbers and a reduction in the MC degranulation product MMCP-1 in serum. Moreover, mice that are unable to produce IgE were also protected against oxazolone-induced colitis. Next, the authors showed that IgE antibodies were produced by B cells in response to IL-13 released by $CD4^+$ Th2 cells. Along the same line, Rijnierse et al. provided evidence that mast activation by immunoglobulin-free light chains (IgLCs) of immunoglobulins are involved in DNBS colitis [28]. Treatment of mice with the IgLC antagonist F991 prevented MC activation and abrogated the development of diarrhea, celllar infiltration and colonic lymphoid follicle hyperplasia. Passive immunization with antigen specific IgLCs and subsequent rectal challenge with the hapten elicited MC activation and increased vascular permeability in the colon of mice. Of note, serum concentrations of IgLCs in CD was increased while more IgLCs were detected in the ileum and colon of patients with IBD [28]. Based on the above, IgE dependent and IgE-independent MC activation may be involved in IBD. Further data in human are however awaited.

Stress, mast cells and IBD

A recent large prospective population-based study in 704 IBD patients identified perceived stress, negative affect or mood and major life events to be associated with symptomatic flares [29]. Knowing that stress is an important trigger of MC activation [30], MC could theoretically be involved in the triggering of these flares, perhaps by increasing permeability thereby exposing the immune system to increased levels of intraluminal antigens. Increased permeability has indeed been reported using biopsies of IBD patients [14] [31], whereas reactivation of colitis by stress was reported already many years ago. This effect was dependent on CD4+ T cells and increased permeability

[32]. In 2009, evidence was reported suggesting that chronic psychological stress, through the release of substance P, triggers the expression and release of corticotrophin releasing hormone by eosinophils. This stress hormone then subsequently activates MC leading to epithelial barrier dysfunction [33]. The above-mentioned studies would suggest that stress-induced flares in IBD may at least partly be explained by MC-mediated changes in barrier function and subsequent reactivation of the immune response.

Summary and conclusion

From the studies summarized above, MC seem to be involved both in the acute and more sustained inflammatory response, at least in preclinical models of IBD. As shown in Figure 1, MC can be activated by stress via eosinophil-derived CRH, IgLC, IgE or ATP leading to the release of a wide variety of mediators, including proteases, histamine, chemokines and cytokines, leading to the attraction of inflammatory cells, changes in barrier function, tissue remodeling etc. Increased permeability may contribute to increased influx of intraluminal antigens either activating MC or CD4+ T cells further contributing to the inflammatory response. This process can be antagonized by inhibition of MC activation via ceramide binding to the inhibitory receptor LIMR3. To what extent this also applies to IBD patients and to what extent MC are indeed a target for treatment remains to be proven. Clearly, the most convincing evidence will have to come from clinical studies evaluating new compounds in patients, however, data from randomized clinical trials are currently available. Nevertheless, based on the data summarized here, protease inhibitors[23,34] and ceramide[21] may represent interesting targets hopefully improving treatment of IBD patients.

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Legend to Figure 1

Schematic representation of the possible involvement of MC in IBD. For more detailed information, see main text.

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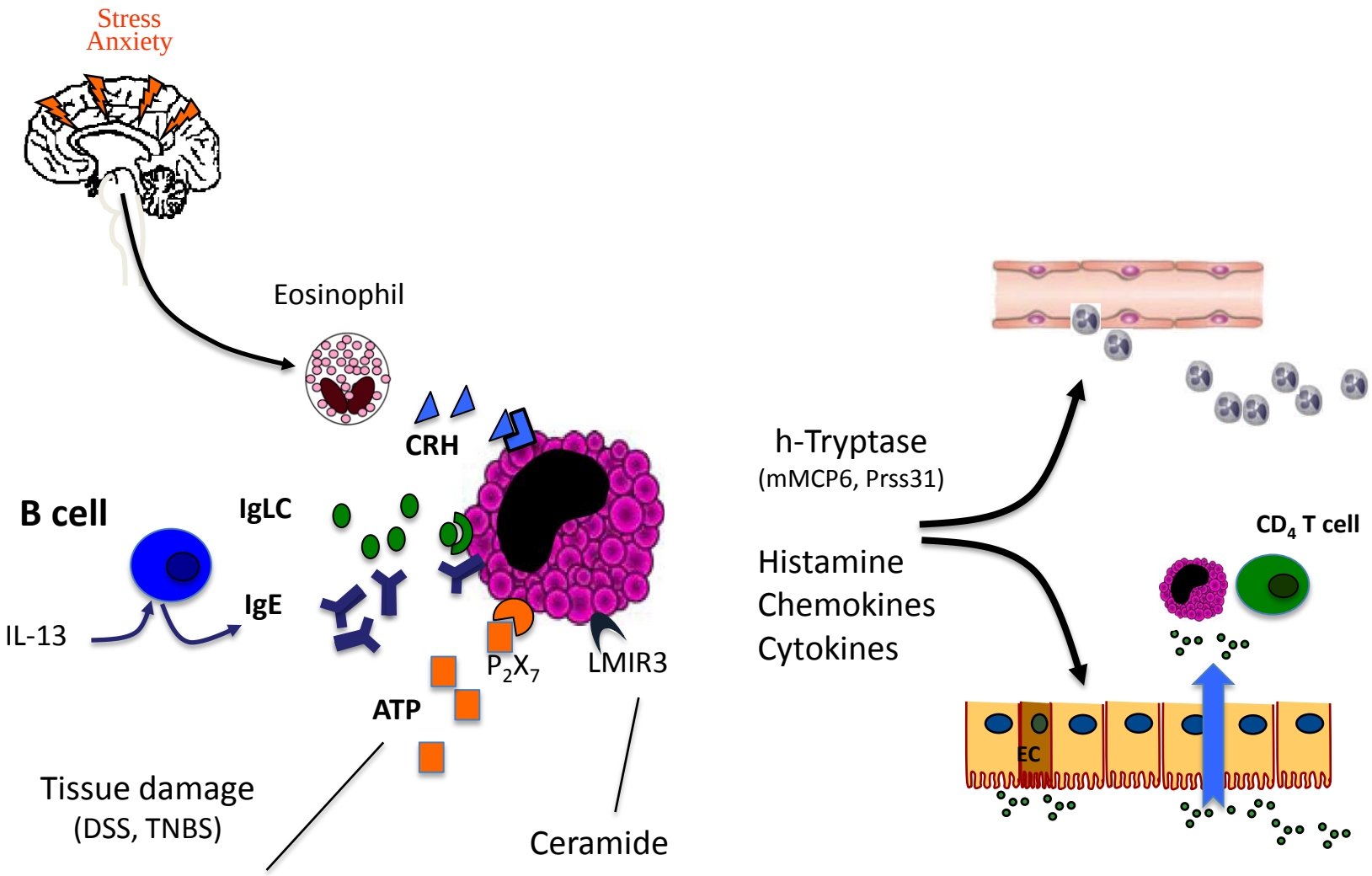
Figure 1


Stress
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Ceramide
h-Tryptase
(mMCP6, Prss31)
Histamine
Chemokines
Cytokines
CD_4 T cell
EC